WU-NK-101: an Enhanced NK Cell Therapy Optimized for Function in the Tumor Microenvironment (TME)

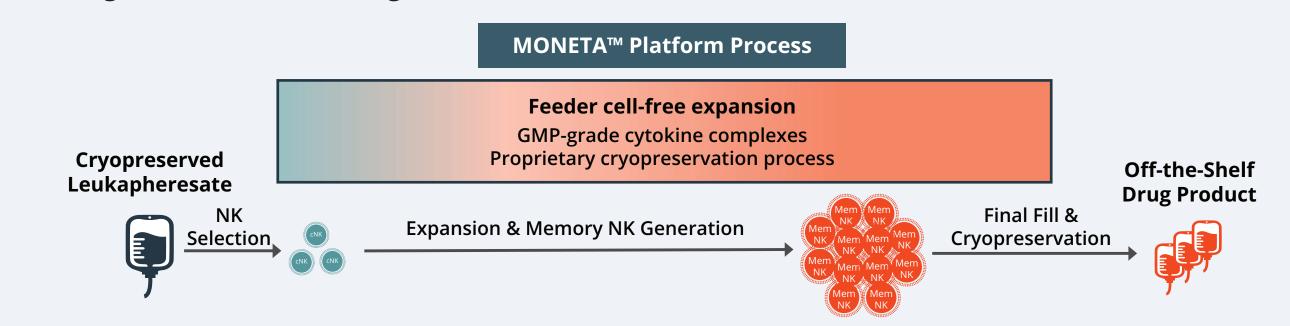
Sergio Rutella*5, John J Muth¹, Mary Elizabeth Mathyer¹, Tom A Leedom¹, Alun Carter¹, Kristann Magee¹, David Boocock⁵, Melissa Berrien-Elliot², Brunda Tumala¹, Mark Foster², Julian Gorrochategui³, Paula Comune Pennacchi³, Daniel Primo³, Juan Ballesteros³, Vladimir Tolstikov⁴, Michael A. Kiebish⁴, Punit Shah⁴, Jayakumar Vadakekolathu⁵, Jan E Baughman⁶, Todd A Fehniger², Matthew L Cooper¹, Jan Davidson-Moncada¹

> ¹Wugen, St Louis, MO, United States of America; ²Washington University St Louis, St Louis, MO, USA; ³Vivia Biotech, Madrid, Spain, ⁴BERG Health, Framingham, MA, USA; ⁵Nottingham Trent University, Nottingham, UK; ⁶Alviso Clinical Research, San Francisco, CA, USA

sergio.rutella@ntu.ac.uk

Background

- Natural Killer (NK) cells, identified as CD3^{neg}/CD56^{pos} or CD3^{neg}/CD56^{dim}/CD16^{pos} lymphocytes, exist in peripheral blood, bone marrow, spleen, lymph nodes
- NK cells are functionally defined as cytotoxic and/or cytokine secreting in response to tumor cells
- NK cells can identify and eliminate virus-infected cells and tumor cells without prior sensitization
- The efficacy of adoptive cell therapies (ACTs) against solid tumors has been limited by identification of target antigens, restricted trafficking to tumors, and establishment of a highly immuno-suppressive tumor microenvironment (TME), aiding in tumor escape and progression
- Efforts are being focused on enhancing NK metabolic fitness and anti-tumor function within a nutrientrestrictive TME
- WU-NK-101, manufactured using the MONETA™ platform, is an ACT product derived from healthy-donor (CD3^{neg}/CD56^{pos}) NK cells to provide enhanced cytotoxicity and metabolic adaptability, addressing current challenges of ACT in the setting of an adverse TME

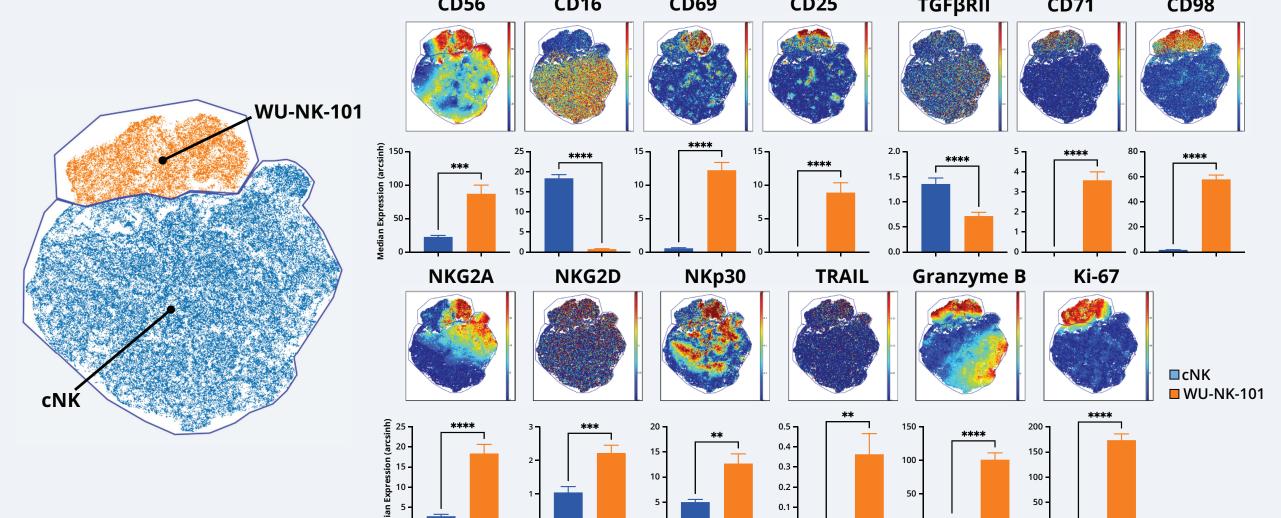


Methods

- WU-NK-101 cells were phenotypically characterized using multidimensional mass cytometry (CyTOF)
- Cytotoxicity was assessed in vitro in 2 culture conditions, complete media (N media; IMDM medium [glucose 25 mM, neutral pH 7.2–7.4]) supplemented with 20% fetal bovine serum (FBS) and antibiotics, and TME-aligned media. TME-aligned media is composed of custom RPMI (pH 6.9, glucose 6 mM) supplemented with human serum, human platelets lysate, antibiotics and cytokines from TNFα stimulated human mesenchymal stem cells (hMSC) culture. Stimulated MSCs secrete soluble molecules, such as nitric oxide, PGE2, IDO, IL-10 and TGF81.^{1,2,3}
- Multi-omic studies were performed by mass spectrometry-based proteomics
- In vitro intrinsic and antibody-dependent cellular cytotoxicity (ADCC) killing assays (IncuCyte, Sartorius): WU-NK-101 cells were co-cultured with tumor cells and vehicle, isotype control antibodies, or trastuzumab over 72 hours
- In vivo: SKOV3-CBR-GFP cells were inoculated (IP) in NSG mice. Mice were then injected with WU-NK-101 cells + trastuzumab or isotype control
- Cell trafficking/penetration to TME was measured in NSG mice (SKOV-3) by tracking fluorescently-labeled WU-NK-101 cells ± trastuzumab to enhance killing of trastuzumab-targeted cells
- Metabolic fitness was assessed by Seahorse Real-Time Cell Metabolic Analysis (Agilent)

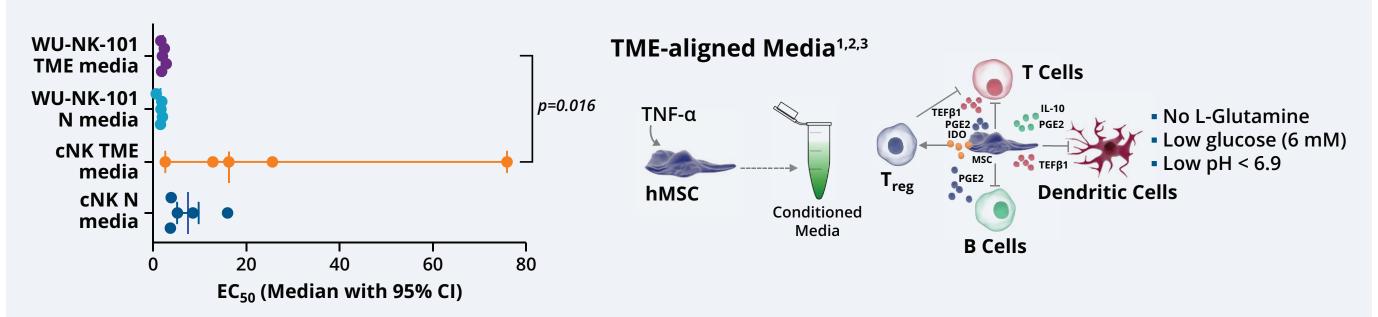
Results

WU-NK-101 Has a Unique Phenotype Optimized for Rapid Activation and Improved Cytotoxicity



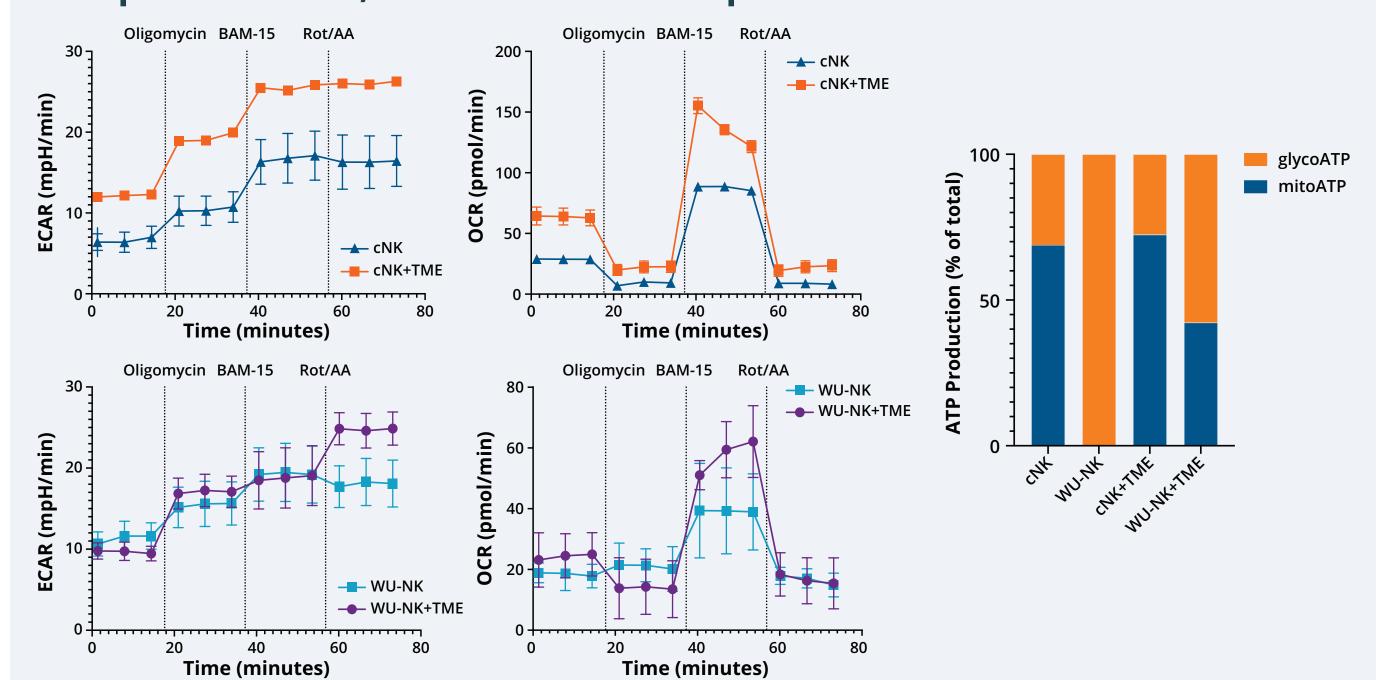
Paired conventional NK cells (cNK) and WU-NK-101 cells were generated from the same donor. Cells were assessed for their phenotype by CyTOF. Briefly, cells were thawed from cryopreservation and then stained with antibodies for surface and intracellular markers. NK cells were run on a Helios mass cytometer and assessed using FlowSOM clustering on CD11b, CD16, CD19- CD3- CD14- (LIN); CD45, CD56, GzmB. NK cells were identified across 3 metaclusters as LIN- CD56^{pos}, CD16^{+/-}, CD11b^{dim/-} (Paired t-test, *<0.05, **<0.01, ***<0.001, ****<0.0001).

WU-NK-101 Cytotoxicity is Not Hampered by Adverse TME as Compared to cNK Cells



Conventional NK cells (CD3^{neg}/CD56^{pos}) and paired WU-NK-101 cells were generated from healthy donors (n=5) and co-cultured with HL-60 cells at various E:T ratios for 48 hours. Percent viable was normalized to control (no NK added).

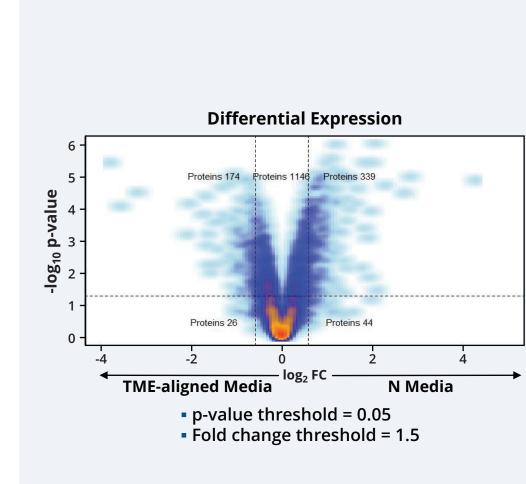
Compared to cNK, WU-NK-101 Has Improved Metabolic Fitness in TME



Mean ± SEM; Oligomycin: inhibits ATP synthase, pushing cells towards glycolysis; BAM15: mitochondrial uncoupler; Rot/AA (roterone/antimycin A): shuts off mitochondrial OCR.

- WU-NK-101 exhibited enhanced glycolytic and mitochondrial oxidative phosphorylation capacity (i.e., metabolic fitness) compared to cNK cells
- WU-NK-101 had metabolic profile consistent with aerobic glycolysis ("Warburg metabolism"), which may abrogate the adverse effects of an inhibitory TME
- Compared to cNK, WU-NK-101 extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were not greatly impacted by the TME

KEGG Pathway Analysis Suggests Metabolic Flexibility of WU-NK-101



Enriched Metabolic Pathways – TME-aligned Media	Adjusted p-value
Steroid biosynthesis	0.0019
Folate biosynthesis	0.0047
Amino sugar and nucleotide sugar metabolism	0.0049
Metabolic pathways	0.0051
Alanine, aspartate and glutamate metabolism	0.0087
D-Glutamine and D-glutamate metabolism	0.0109
Nitrogen metabolism	0.0320

Enriched Metabolic Pathways – N Media	Adjusted p-value
Pentose phosphate pathway	0.0003
Glycolysis / Gluconeogenesis	0.0020
Amino sugar and nucleotide sugar metabolism	0.0310
Metabolic pathways	0.0339
Fructose and mannose metabolism	0.0339

■ Metabolic flexibility aids in function within the TME. In N media, WU-NK-101 used glucose as its main nutritional source; in hypoglycemic TME-aligned media, amino acid metabolic pathways were upregulated, which augurs metabolic adaptability

and Persistence s.c. SKOV-3-bearing **Tumor Localization NSG Mouse Resected Tumors Far-Red In Vivo Dye Labeling**

8 hours

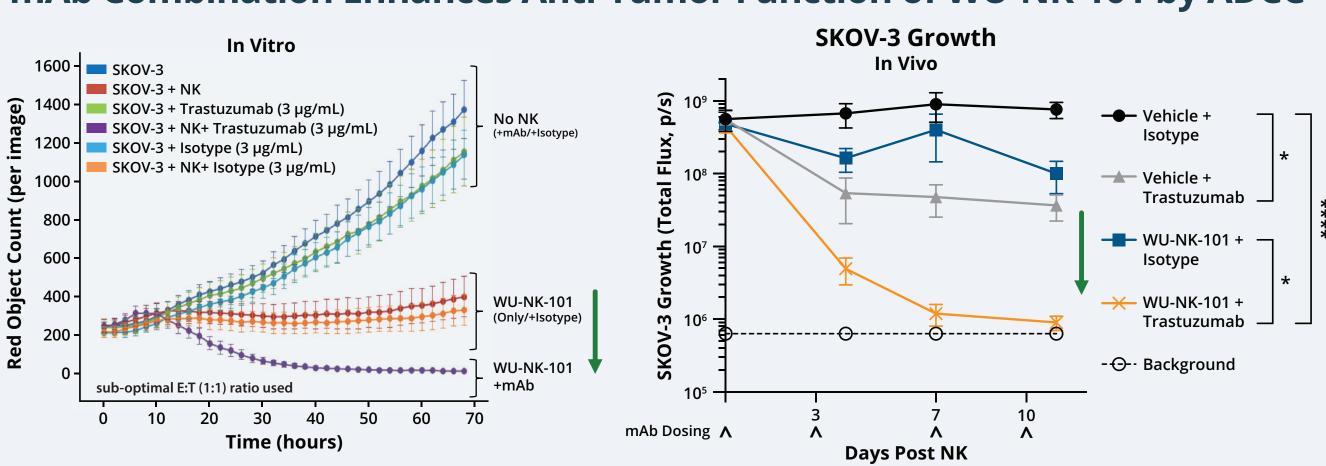
24 hours

2×10¹⁰

WU-NK-101 in Combination with mAb Increases Intra-Tumor Infiltration

- WU-NK-101 trafficking is further enhanced with mAb (trastuzumab) dosing (top panel)
- Sequential dosing of mAb followed by WU-NK-101 (24 hrs) further improves WU-NK-101 penetration (left) and persistence in tumors (right) (bottom panel)

mAb Combination Enhances Anti-Tumor Function of WU-NK-101 by ADCC



WU-NK-101 intrinsic and ADCC killing assays in vitro (left panel): Tumor cells were were co-cultured with WU-NK-101 cells and isotype control antibodies or trastuzumab. WU-NK-101 intrinsic killing and ADCC was assessed over 72 hours using IncuCyte. In vivo (right panel): SKOV-3-CBR-GFP cells were inoculated (IP) in NSG mice on Day -3. Mice were randomized on Day 0 and were injected with WU-NK-101 cells $(1x10^7, IP)$ + trastuzumab or isotype.

Conclusions

- WU-NK-101 exhibited enhanced/adaptive metabolic fitness contributing to resilience to an adverse, highly-immunosuppressive TME, relative to cNK cells
- WU-NK-101 showed potent cytotoxicity against tumor cells in vitro and in vivo, which could be further enhanced by ADCC
- WU-NK-101 in combination with mAb enhanced trafficking and tumor penetration, contributing to anti-tumor activity
- These data suggest that WU-NK-101 may overcome current limitations of adoptive cellular therapies and support clinical evaluation of NK cell-based approaches in the setting of solid tumors

References

1. Samsonraj et al. *Stem Cells Transl Med.* 2017 Dec;6(12):2173-2185. **2.** Sasser et al. *Cancer Lett.* 2007 Sep 8;254(2):255-64. **3.** Studebaker et al. *Cancer Res.* 2008 Nov 1;68(21):9087-95.

Conflict of Interest

Sergio Rutella's lab at Nottingham Trent University received research support from Wugen, Inc.

Copies of this poster obtained through QR (Quick Response) and/or text key codes are for personal use only and may not be reproduced without written permission of the authors.



72 hours